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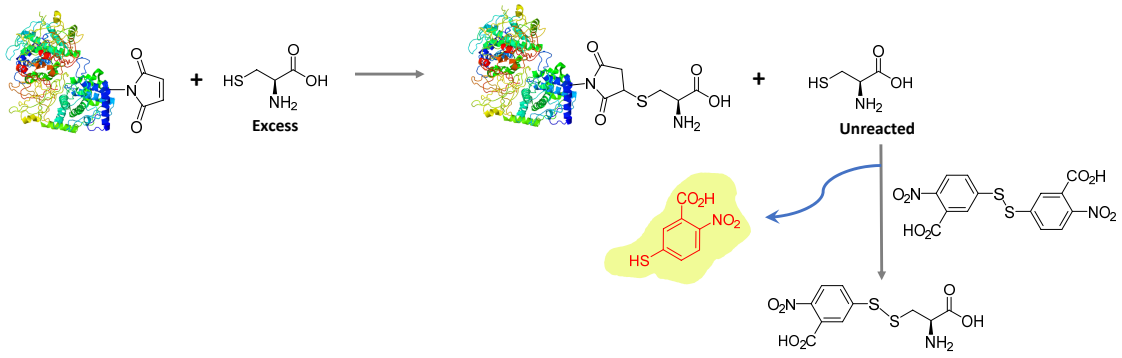
Maleimide Assay Kit

Product Number: **CM90002**

Product Description

CellMosaic's maleimide assay kit is designed to assay the maleimide functional group content of a modified biopolymer, such as an antibody, protein, peptide, or oligo. CellMosaic routinely uses this kit for its internal bioconjugation-related research.

The assay is based on Ellman's assay of cysteine using 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) (Ellman, G.L. 1959, Tissue sulfhydryl groups. *Arch Biochem Biophys.* 82, 70–77). First, a known amount of excess cysteine is reacted with the maleimide groups of the biopolymer, and then the unreacted cysteine is reacted with DTNB to generate 2-nitro-5-thiobenzoic acid (TNB) (**Scheme 1**). TNB is orange in color and has an extinction coefficient of 14,150 $M^{-1}cm^{-1}$ at 412 nm (Riddles, P.W. Blakeley, R. L., and Zerner, B. 1983, Reassessment of Ellman's reagent. *Methods Enzymol.* 91, 49-60). The difference between the initial amount of cysteine and the amount of unreacted cysteine corresponds to the maleimide functional groups of the biopolymer.



Scheme 1: Principle of Maleimide Assay (CM90002)

Application of the Product

- Assay the maleimide functional groups.

Key Features of the Product

- Less than 1 h of preparation and assay time. Fast and easy to use.

Kit Components

Four micro-centrifuge tubes per package. Each package is sufficient for 10 assays (100 µL per assay volume)

Name	Part #	Quantity
Cysteine (pink label)	CM13008	1 unit
DTNB (orange label)	CM13005	1 unit
Buffer A (indigo label)	CM02005	1 mL
Solution A (green label)	CM01003	1 mL

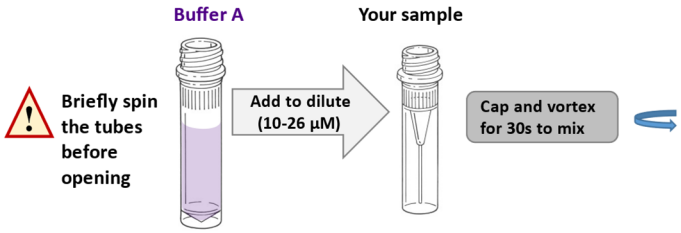
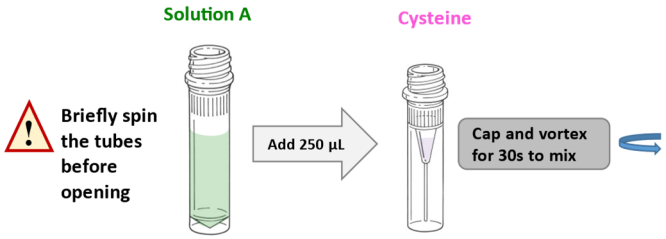
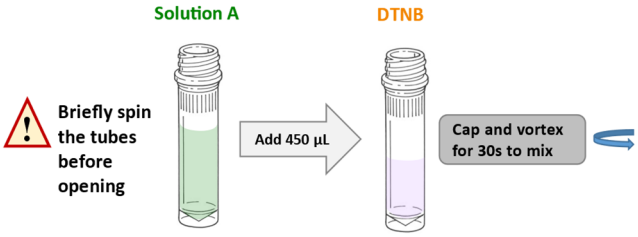
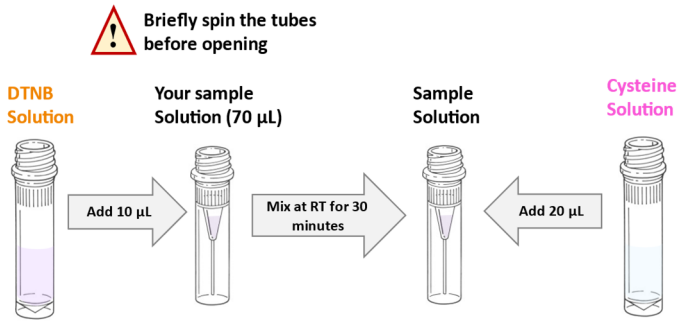
Storage/Stability

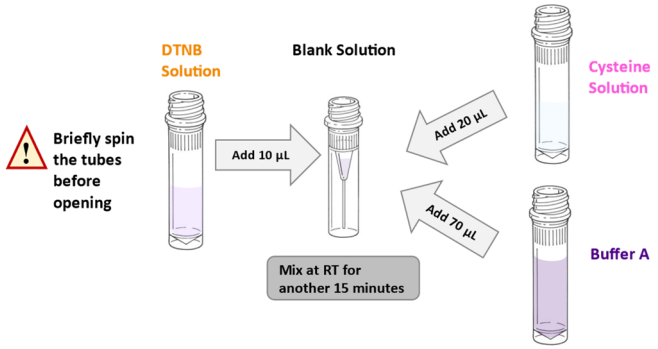
Recommended storage of the kit is at 2-8°C. For cysteine and DTNB dissolved in solution A, they can be aliquoted and stored at -20°C up to 1 year without any sign of decomposition.

Equipment (not provided)

1. UV/vis spectrophotometer or micro-plate reader spectrophotometer with pathlength correction capability
2. Ultra-micro UV transparent cuvette with 1 cm path length: 100 µL (for UV/vis spectrophotometer) or 96-well UV microplate
3. Two clean 0.5 mL microcentrifuge tube for preparing sample solution and blank solution

Protocol

1. Sample preparation: Briefly spin the tube containing **Buffer A** (indigo label) before opening it. Dilute the biopolymer in **Buffer A** to a total volume of **70 µL** and a final concentration of maleimide groups in the 10–26 µM range. **Note**: If it is an antibody with an average 4 maleimide groups per antibody, you can dilute to 0.4-1 mg/mL).	
2. Prepare Cys solution: Briefly spin the tube containing **Cysteine** (pink label) and **Solution A** (green label) before opening the tubes. Pipette 250 µL of **Solution A** into the **Cysteine tube**. Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap.	
3. Prepare DTNB solution: Briefly spin the tube containing **DTNB** (orange label) before opening it. Pipette 450 µL of **Solution A** into the **DTNB tube.** Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap.	
4. Prepare sample solution: Mix 70 µL of sample with 10 µL of **Cys solution** at RT for 30 minutes, then add 20 µL of **DTNB solution** and mix at RT for another 15 minutes.	

5. Prepare blank solution: mix 70 µL of **buffer A**, 10 µL of **Cys solution**, and 20 µL of **DTNB solution** and mix at RT for 15 minutes. **Note:** Aliquot and store the rest of the **Cys and DTNB solution** at -20°C for later usage.	
6. UV reading: Measure the UV absorbance of the sample and blank solution at 412 nm.	As (sample): _____ Ab (blank): _____
7. Calculate the value of maleimide content after dilution in Step 1: $\mu M = 101 \times (Ab - As)$ Cuvette pathlength: 1 cm	µM = _____
8. Calculate the degree of labeling (DOL) based on the following formula: $DOL = \frac{\mu M\ (Maleimide)}{\mu M\ (Biopolymer)}$ Where µM (Biopolymer) is the concentration of biopolymer after dilution in Step 1.	DOL = _____

Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

The information provided in this document and the methods included in this package are for information purposes only. CellMosaic provides no warranty of performance or suitability for the purpose described herein. Information about the chemicals and reagents used in the kit are provided as necessary.

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